

## STABLE TABLET FORMULATION

## CROSS-REFERENCE TO RELATED APPLICATION

This is the U.S. national phase application of International Application No. PCT/US2005/041252, filed Nov. 16, 2005, which claims the benefit of U.S. Provisional Application No. 60/629,189, filed Nov. 17, 2004.

## BACKGROUND

## 1. Field

The present invention is generally directed to stable tablet formulations of tetrahydrobiopterin or precursors, derivatives or analogs thereof for the treatment of humans.

## 2. Background of the Related Technology

Tetrahydrobiopterin (sometimes referred to as BH4) is a biogenic amine of the naturally-occurring pterin family that is a cofactor for a number of different enzymes, including phenylalanine hydroxylase (PAH), tyrosine hydroxylase, tryptophan hydroxylase and nitric oxide synthase. Pterins are present in physiological fluids and tissues in reduced and oxidized forms, however, only the 5,6,7,8, tetrahydrobiopterin is biologically active. It is a chiral molecule and the 6R enantiomer of the cofactor is known to be the biologically active enantiomer. For a detailed review of the synthesis and disorders of BH4 see Blau et al., 2001 (*Disorders of tetrahydrobiopterin and related biogenic amines*. In: Scriver C R, Beaudet A L, Sly W S, Valle D, Childs B, Vogelstein B, eds. *The Metabolic and Molecular Bases of Inherited Disease*. 8th ed. New York: McGraw-Hill, 2001: 1275-1776).

## Names of Parties to a Joint Research Agreement

BioMarin Pharmaceutical Inc. and Merck Eprova AG

A deficiency in PAH activity, due to absent or mutated PAH enzyme or a deficiency in its cofactor BH4, manifests as an excess of the amino acid phenylalanine (Phe) known as hyperphenylalaninemia (HPA) in its very mildest forms or phenylketonuria (PKU) in its moderate or severe forms. PAH deficiency also causes a deficiency in the amino acid tyrosine, which is a precursor for synthesis of neurotransmitters. A deficiency in tyrosine hydroxylase or tryptophan hydroxylase activity can manifest as a deficiency in neurotransmitter production.

Despite the elucidation of the role of BH4 deficiency in phenylketonuria, treatment with BH4 has not been suggested because such treatment is very expensive, as high as \$30,000 per year for an adolescent or adult, as compared with \$6,000 for phenylalanine-restricted dietary therapy (Hanley, *N. Engl. J. Med* 348(17):1723, 2003). Another significant problem with BH4 is that this compound is unstable and readily undergoes aerobic oxidation at room temperature (Davis et al., *Eur. J. Biochem.*, Vol 173, 345-351, 1988; U.S. Pat. No. 4,701, 455) and has a shelf-life of less than 8 hours at room temperature (Bernegger and Blau, *Mol. Genet. Metabol.* 77:304-313, 2002).

Other tetrahydrobiopterin products available on the market need to be specially packaged or kept frozen. For example the labeling on the tablets sold by Schirck's Laboratory specify that the tablets should be kept frozen and state that the product has a shelf life at room temperature of only 2 months. BIOPTEN (tetrahydrobiopterin granules) requires expensive, hermetically-sealed foil packaging to maintain room temperature stability. The instability of such BH4 compositions is

commercially undesirable and significant degradation due to improper storage could hinder therapy of patients.

Drug substance polymorphic forms can exhibit different physical and mechanical properties, including hygroscopicity, particle shape, density, flowability, and compactibility, which in turn may affect processing of the drug substance and/or manufacturing of the drug product. The effect of polymorphism on pharmaceutical processing also depends on the formulation and the manufacturing process. Polymorphic forms of the drug substance can undergo phase conversion when exposed to a range of manufacturing processes, such as drying, milling, micronization, wet granulation, spray-drying, and compaction. Exposure to environmental conditions such as humidity and temperature can also induce polymorph conversion. The extent of conversion generally depends on the relative stability of the polymorphs, kinetic barriers for phase conversion, and applied stress. See FDA Center for Drug Evaluation and Research (CDER) Draft Guidance for Industry ANDAs: Pharmaceutical Solid Polymorphism Chemistry, Manufacturing, and Controls Information, December 2004.

Thus, there remains a need for a stable solid formulation of tetrahydrobiopterin and processes for manufacturing such stable formulations. The present invention is directed to addressing such a need.

## SUMMARY OF THE INVENTION

The present invention relates to stable solid formulations of tetrahydrobiopterin, particularly stable tablets, processes for producing such formulations, and treatment methods using such formulations.

The invention provides a stable solid formulation of tetrahydrobiopterin, or a precursor or derivative or analog thereof, that maintains its stability for an extended period of time. Compositions of the invention may comprise a stable, crystalline form of BH4 that is stable at room temperature for more than 8 hours and a pharmaceutically acceptable carrier, diluent or excipient. Exemplary stable tablets of the invention have been prepared using a dry tableting process and have been shown to have a shelf-life of at least 6 to 9 months at room temperature.

Another aspect of the invention provides a dry formulation process for preparing stable solid formulations, which includes the step of mixing tetrahydrobiopterin, or a precursor or derivative or analog thereof, with another pharmaceutical carrier, diluent or excipient, in the absence of added water.

In an exemplary embodiment, the active pharmaceutical ingredient and excipients are dry blended and compressed. The tablets are processed in humidity-controlled rooms where humidity is kept at about 65% ( $\pm 5\%$ ) or less. Once processed, the tablets are stored in triple plastic lined water resistant containers with desiccant pillows lined between the outer two layers of plastic bags. Thus, the invention includes a dry formulation method comprising the steps of mixing an initial amount of a crystalline polymorph of (6R)-L-erythro-tetrahydrobiopterin, preferably polymorph B, and one or more pharmaceutically acceptable excipients, and forming a tablet from the mixture, wherein the steps do not include adding liquid water. Exemplary particle sizes include, e.g., from about 0.2  $\mu\text{m}$  to about 500  $\mu\text{m}$ , from about 1  $\mu\text{m}$  to about 250  $\mu\text{m}$ , or from about 2  $\mu\text{m}$  to about 200  $\mu\text{m}$ , or, e.g., smaller than about 500  $\mu\text{m}$ , smaller than about 600  $\mu\text{m}$ , smaller than about 700  $\mu\text{m}$ , or smaller than about 850  $\mu\text{m}$ .

In exemplary embodiments, the tablet is initially manufactured using a stable crystalline form of (6R)-5,6,7,8-tetrahy-